10-1992

New Distribution Records for *Bunodera eucaliae* and *Bunodera inconstans* n. comb. (Digenea: Allocreadiidae: Bunoderinae) with Discussion of Their Phylogenetic Relationships

Daniel R. Brooks
University of Toronto, dnlbrooks@gmail.com

J. Parasitol., 78(5), 1992, p. 779–783

NEW DISTRIBUTION RECORDS FOR *BUNODERA EUCALIAE* AND *BUNODERA INCONSTANS* N. COMB. (DIGENEA: ALLOCREADIIDAE: BUNODERINAE) WITH DISCUSSION OF THEIR PHYLOGENETIC RELATIONSHIPS

Daniel R. Brooks
Department of Zoology, University of Toronto, Toronto, Ontario, Canada M5S 1A1

ABSTRACT: *Bunodera eucaliae* inhabits *Culaea inconstans* from eastern Ontario and western Nebraska, both new geographic distribution records. *Culaeatrema inconstans* inhabits *Culaea inconstans* from eastern Ontario, also a new distribution record. Comparison of *Culaeatrema inconstans* with species of *Paracreptotrematina, Bunoderella, Bunodera,* and *Crepidostomum* within a phylogenetic context suggests that it is the sister species of *B. eucaliae.* Accordingly, it is transferred to *Bunodera,* although the monophyly of *Bunodera* as a group has not yet been established because there is no synapomorphy linking *Bunodera luciopercae* with the clade containing *Bunodera mediovitellata, Bunodera sacculata, B. eucaliae,* and *B. inconstans.*

As part of a study of possible effects of parasite load on vigor of mating behavior, I collected helminth parasites from 100 specimens of brook stickleback, *Culaea inconstans* (Kirkland), from Tooley Creek, near Darlington, Ontario, during late May and early June 1991. Among the parasites collected were numerous specimens of *Bunodera eucaliae* (Miller, 1936) Miller, 1940 (Digenea: Allocreadiidae), which inhabited 75 (75%) of the sticklebacks examined. I subsequently collected specimens of another allocreadiid digenean species, *Culaeatrema inconstans* Lasee, Font, and Sutherland, 1988, in 2 of 10 (20%) *Culaea inconstans* from Lake Sasajewan, Algonquin Park, Ontario, during early June 1991 and specimens of *B. eucaliae* in 7 of 20 (35%) *Culaea inconstans* from the Snake River, 30 km south of Merriman, Cherry County, Nebraska, during late July 1991. This study was prompted by the pronounced similarity between *B. eucaliae* and *Culaeatrema inconstans,* both hosted by *Culaea inconstans.*

MATERIALS AND METHODS

Digeneans were removed alive from their hosts, washed in saline, flattened with slight coverslip pressure, and fixed with alcohol–formalin–acetic acid (AFA). They were stored in AFA for 24 hr, then transferred to 70% EtOH. Whole mounts were stained with Mayer's hematoxylin and mounted in Canada balsam. In addition to specimens collected in this study and deposited in the Harold W. Manter Laboratory, Division of Parasitology, University of Nebraska State Museum (HWML) as voucher specimens (HWML no. 34099 from Tooley Creek, Ontario; HWML no. 35089 from the Snake River; HWML no. 35088 from Algonquin Park, Ontario), I examined 4 paratypes of *Culaeatrema inconstans* (USNM Helm. Coll. nos. 78395 and 78396).

Phylogenetic analyses were performed manually using Hennig argumentation procedures (Brooks and McLennan, 1991; Wiley et al., 1991), and results were checked using the PAUP 3.0 computer program run on a Macintosh IIX computer.

RESULTS

Culaea inconstans has been reported as host for 3 species of bunoderine allocreadiids, *Bunodera sacculata* Van Cleave and Mueller, 1936, *B. eucaliae,* and *Culaeatrema inconstans. Bunodera sacculata* is geographically widespread in North America, having been reported from Quebec, Ontario, Connecticut, Delaware, Michigan, New York, Wisconsin, and Iowa. The species has been reported in *Culaea inconstans* only once, being more commonly reported in percid, cyprinid, and centrarchid fishes (Caira, 1989). *Bunodera eucaliae,* which was described originally inhabiting *Culaea inconstans* from Jacques Cartier County, Quebec, is also geographically widespread, having been found in Oregon, Washington, and British Columbia, where it inhabits primarily *Gasterosteus aculeatus* but it also has been found in mud minnows (Umbriidae) and in Iowa, Wisconsin, and Ontario, where it has been reported only in *Culaea inconstans* (see Caira, 1989). By contrast, *Culaeatrema inconstans* has been reported only in *Culaea inconstans* and only from Wisconsin (Lasee et al., 1988). Comparison of specimens collected from Tooley Creek and Lake Sasajewan, Ontario, and from the Snake River, Nebraska, with Caira's (1989) revision of the papillose allocreadiids (Bunoderinae Looss, 1902 sensu Yamaguti, 1971) and with paratype

Received 12 February 1992; revised 3 June 1992; accepted 8 June 1992.

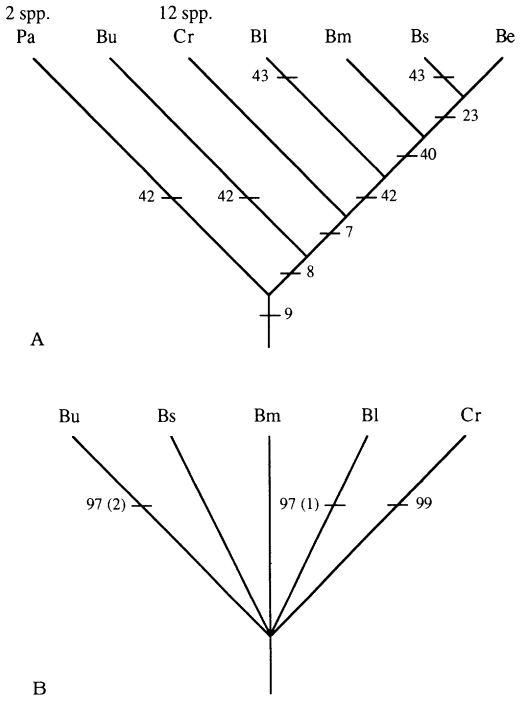

FIGURE 1. Phylogenetic trees for bunoderine digeneans. **A.** Phylogenetic tree for *Paracreptotrematina* (Pa), *Bunoderella* (Bu), *Crepidostomum* (Cr), *Bunodera luciopercae* (Bl), *Bunodera mediovitellata* (Bm), *Bunodera sacculata* (Bs), and *Bunodera eucaliae* (Be) based on adult characters (redrawn and modified from Caira [1989] to show only single branches for *Paracreptotrematina* [with 2 spp.] and *Crepidostomum* [with 12 spp.]). **B.** Phylogenetic tree for *Bunoderella metteri*, 4 species of *Crepidostomum*, and 3 species of *Bunodera* based on larval characters (redrawn and modified from Caira [1989]). Numbers accompanying slash marks indicate apomorphic states for characters used by Caira (1989).

material of *Culaeatrema inconstans* supports the conclusion that the specimens collected from Tooley Creek and from western Nebraska are members of *B. eucaliae,* whereas those collected from Algonquin Park are members of *Culaeatrema inconstans.* Tooley Creek, Ontario, and the Snake River, Cherry County, Nebraska, constitute new geographic distribution records for *B. eucaliae*; Lake Sasajewan, Algonquin Park, Ontario, represents a new geographic distribution record for *Culaeatrema inconstans.*

I did not find any specimens of *B. eucaliae,* or any other allocreadiid species, in 40 *Culaea inconstans* collected from populations near Kingsley Dam, 5 km east and 11 km north of Ogallala, Keith County, Nebraska, or 30 *Culaea inconstans* collected 2 km south of Sutherland, Lincoln County, Nebraska, localities that occur within 65 km of the Snake River populations. The localities from which allocreadiids were not collected are among the southernmost for populations of *Culaea inconstans* and occur in the Platte River system, which drains eastward into the Missouri River, whereas the Snake River drains northward into the Missouri River.

DISCUSSION

Lasee et al. (1988) proposed the name *Culaeatrema inconstans* for specimens of an allocreadiid digenean species collected from the intestines of *Culaea inconstans* from Wisconsin. Lasee et al. (1988) recognized marked similarity between *Culaeatrema inconstans* and members of the subfamily Bunoderinae (postovarian uterine loops, ceca extending posteriorly to the level of the testes, oblique testes, and vitellaria not extending posteriorly to the posterior end of the body), but they proposed a new genus within the subfamily to accommodate the species because specimens lacked the oral papillae diagnostic for the rest of the members of the Bunoderinae. They also reported finding 2 apparently parthenogenetic populations of the species, comprising ovigerous specimens that lacked 1 or both testes and in which no sperm could be detected in the seminal vesicle. In this study, 5 of the 7 specimens of *Culaeatrema inconstans* collected in *Culaea inconstans* from Lake Sasajewan lacked testes and 2 had 1 testis only. In none of the specimens was sperm evident in the seminal vesicle. This suggests that parthenogenesis in this species is widespread geographically.

Caira (1989) revised the papillose allocreadiids and included a phylogenetic analysis based on adult and larval characters, completing her study prior to the description of *Culaeatrema.* Within *Bunodera,* Caira (1989) considered *B. eucaliae* and *B. sacculata,* which have been reported in *Culaea inconstans* in Wisconsin (see Caira, 1989), to be sister species, based on their shared possession of ceca extending posteriorly only to the mid-hindbody, a synapomorphic condition for those 2 species, and the possession of vitellaria extending posteriorly only to the mid-hindbody, a synapomorphic trait linking *B. sacculata* + *B. eucaliae* with *B. mediovitellata* Tsimbaliuk and Roitman, 1966 (Fig. 1). *Culaeatrema inconstans* possesses both of the synapomorphic traits for the *Bunodera mediovitellata–B. sacculata–B. eucaliae* and the *B. sacculata–B. eucaliae* clades. It furthermore closely resembles *B.*

eucaliae and differs from *B. sacculata* and *B. mediovitellata* by having an acetabulum that is wider than the oral sucker and testes positioned at or posterior to the posterior ends of the ceca. Cannon (1971) reported a population of parthenogenetic, apparently triploid, *B. sacculata* in Algonquin Park, Ontario. If *B. sacculata, B. eucaliae,* and *Culaeatrema inconstans* form a clade, parthenogenetic populations of *B. eucaliae* might be expected; to my knowledge, this has not yet been documented.

Based on the above morphological similarities and the fact that both species inhabit *Culaea inconstans,* it appears that if *Culaeatrema inconstans* possessed oral papillae, it would be considered the sister species of, or perhaps even conspecific with, *B. eucaliae.* In this light, it is pertinent to note that the oral papillae in *B. eucaliae* are relatively much smaller than in any other species of Bunoderinae examined and discussed by Caira (1989). The nonbasal placement of *B. eucaliae* in the phylogenetic tree presented by Caira (1989) precludes the interpretation that the small papillae exhibited by *B. eucaliae* are primitive precursors to the larger papillae exhibited by other papillose allocreadiids. Consequently, their condition is interpreted as apomorphic, implying an evolutionary reduction in size. Figure 5 in the original description of *Culaeatrema inconstans* by Lasee et al. (1988) is a scanning electron photomicrograph of the oral region of a specimen of *Culaeatrema inconstans* showing 2 lateral pads on the oral sucker. The case for considering *Culaeatrema inconstans* the sister species of *B. eucaliae* would be strengthened further if those pads represent vestigial oral papillae, demonstrating additional evolutionary reduction in a lineage that already shows reduction in the size of the oral papillae.

Figure 1A is the phylogenetic tree presented by Caira (1989) for the genera of the Bunoderinae based on adult characters. This suggests that *Paracreptotrematina* Amin and Myer, 1982, *Bunoderella* Schell, 1964, and *Bunodera* are monophyletic groups, but that *Crepidostomum* Braun, 1900, is not. Figure 1B is a partial reproduction of the phylogenetic tree presented by Caira (1989) for *Bunoderella metteri* Schell, 1964, 3 species of *Bunodera* and 4 species of *Crepidostomum* based on larval characters (Fig. 1B shows only a single branch for the *Crepidostomum* species). This provides a synapomorphy (character 97[2] of Caira [1989]) supporting the monophyly of *Bunoderella* (which is monotypic) and a possible synapomorphy (character 99 of Caira [1989]) supporting the monophyly of *Crepidostomum.*

Of concern is the fact that the adult diagnostic trait supporting the monophyly of *Paracreptotrematina, Bunoderella,* and *Bunodera* is the same trait, the presence of posttesticular uterine loops (character 42 of Caira [1989]). Outgroup comparisons using phylogenetic studies of digenean family-level groups and above (Brooks et al., 1985, 1989; Brooks and McLennan, 1993) suggest that posttesticular uterine loops should be considered plesiomorphic rather than apomorphic at higher levels within the Digenea, although convergent evolution appears common. Caira (1989) considered posttesticular uterine loops an apomorphic trait within the Bunoderinae because the 9 allocreadiine species she used as outgroups had only preovarian uterine loops. Based on that coding, she then postulated that posttesticular uterine loops evolved independently 3 times, in the ancestor of *Paracreptotrematina,* in *B. metteri,* and in the ancestor of *Bunodera.* Phylogenetic character optimization (Brooks and McLennan, 1991; Wiley et al., 1991) of this character on Caira's tree (Fig. 1A) demonstrates that even if Caira's polarity is adopted, in which case the outgroup node is preovarian uterine loops, it is more parsimonious to say that the ingroup node state is posttesticular uterine loops. That is, posttesticular uterine loops arose no later than in the ancestor of the Bunoderinae and pretesticular uterine loops arose subsequently in the ancestor of *Crepidostomum,* an interpretation that requires 2 evolutionary steps rather than 3; I have therefore reversed the coding for this character in Table I. This change in polarity provides a synapomorphy supporting the monophyly of *Crepidostomum,* but it leaves no synapomorphy supporting the monophyly of *Paracreptotrematina* and *Bunodera.* In the case of *Bunodera,* there is no synapomorphy uniting *Bunodera luciopercae* (Mueller, 1776) Stiles and Hassall, 1898, the type species of *Bunodera,* with the other species of *Bunodera.* Despite these problems with the generic-level groupings, *Culaeatrema inconstans* still remains best explained as the sister species of *B. eucaliae.* This is shown in Figure 2, which is a phylogenetic tree based on the characters described by Caira (1989) and those presented herein, summarized in a data matrix suitable for computer-assisted manipulation in Table I. The phylogenetic tree has a consistency index of 93.3% and differs from that presented by Caira (1989) in only 2 aspects, the lack of synapomorphies for

TABLE I. Data matrix for *Paracreptotrematina* (PA), *Bunoderella metteri* (BU), *Crepidostomum* (CR), *Bunodera luciopercae* (BL), *Bunodera mediovitellata* (BM), *Bunodera sacculata* (BS), *Bunodera eucaliae* (BE), and *Bunodera inconstans* (BI).

	Characters*											
Taxa	1	2	3	4	5	6	7	8	9	10	11	12
PA	1	0	?	0	0	?	0	0	0	0	0	0
BU	1	1	2	0	0	0	0	0	0	0	0	0
CR	1	1	0	1	1	1	0	0	0	0	0	0
BL	1	1	1	1	0	0	1	0	0	0	0	0
BM	1	1	0	1	0	0	0	1	0	0	0	0
BS	1	1	0	1	0	0	1	1	1	0	0	0
BE	1	1	?	1	0	?	0	1	1	1	1	1
BI	1	1	?	1	0	?	0	1	1	1	1	2

* 0, plesiomorphic condition; 1 and 2, apomorphic conditions; ?, missing data (characters for larval stages in species for which no life cycle data are known). Character **1**, ventral papillae absent (0), present (1) (character 9 of Caira [1989]). Character **2**, dorsolateral papillae absent (0), present (1) (character 8 of Caira [1989]). Character **3**, 2 pairs of penetration glands in cercariae (0), 3 pairs of penetration glands in cercariae (1), 4 pairs of penetration glands in cercariae (2) (character 97 of Caira [1989]). The transformation series is 0 → 1 and 0 → 2 (run "unordered" in computer-assisted analyses). Character **4**, dorsomedial papillae absent (0), present (1) (character 7 of Caira [1989]). Character **5**, uterine loops posttesticular (0), pretesticular (1) (character 42 of Caira [1989] recoded as discussed in text). Character **6**, cercarial flame cell formula 2[(3+3+3) + (3+3+3)] (0), 2[(2+2+2) + (2+2+2)] (1) (character 99 of Caira [1989]). Character **7**, ascending ramus of uterus a narrow tube (0), an expanded sac in older individuals (1) (character 43 of Caira [1989]). Character **8**, posterior extent of vitellaria near posterior end of body (0), to mid-hindbody (1) (character 40 of Caira [1989]). Character **9**, ceca long, extending near posterior end of body (0), short, not extending posteriorly to mid-hindbody (1) (character 23 of Caira [1989]). Character **10**, acetabular diameter less than or equal to oral sucker diameter (0), greater than oral sucker diameter (1). Character **11**, testes not positioned near posterior ends of ceca (0), positioned at or just posterior to cecal ends (1). Character **12**, oral papillae large, bases of dorsal papillae touching (0), small, bases of dorsal papillae not touching (1), lacking (2). The transformation series is 0 → 1 → 2 (run "ordered" in computer-assisted analyses).

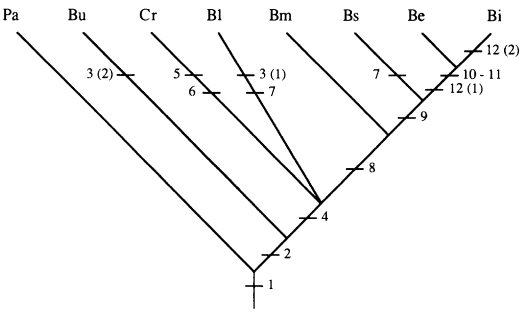

FIGURE 2. Phylogenetic tree for *Paracreptotrematina* (Pa), *Bunoderella metteri* (Bu), *Crepidostomum* (Cr), *Bunodera luciopercae* (Bl), *Bunodera mediovitellata* (Bm), *Bunodera sacculata* (Bs), *Bunodera eucaliae* (Be), and *Bunodera inconstans* (Bi) based on characters discussed in text and described and summarized in Table I. Numbers accompanying slash marks indicate apomorphic states for characters listed in Table I. The consistency index for the tree is 93.3%.

Paracreptotrematina and *Bunodera,* and the inclusion of *Culaeatrema inconstans* as the sister species of *B. eucaliae,* based on their shared possession of the apomorphic conditions for character 10 (acetabular diameter greater than oral sucker diameter) and character 11 (testes positioned at or just posterior to cecal ends). Character 12 concerns the relative sizes of the oral papillae; as indicated in discussion above, the plesiomorphic condition is "large" oral papillae, i.e., those in which the bases of the dorsal papillae touch (see Caira, 1989), whereas "small" oral papillae, i.e., those in which the bases do not touch (see Caira, 1989), are apomorphic. *Bunodera sacculata* and *B. eucaliae,* which have small oral papillae, are the 2 closest relatives of *Culaeatrema inconstans,* which lacks oral papillae. I have signified this, in accordance with the above discussion, as the endpoint in the evolutionary reduction of oral papillae size in this clade, signified as an apomorphic state derived from small papillae.

The possibility of evolutionary loss of characters is potentially troublesome for phylogenetic systematic analysis because secondarily lost traits are indistinguishable, by themselves, from primitively absent traits, even using outgroup comparisons. The current data base for parasitic platyhelminths suggests that approximately 10.8% of the morphological characters analyzed phylogenetically thus far represent cases of evolutionary loss of characters, and nearly half of those represent convergent losses (Brooks and McLennan, 1993). This implies that, although evolutionary losses occur, they are uncommon enough that they should be detectable in any analysis using a relatively large number of characters. In this case, only 1 of 14 apomorphic traits represents a putative case of evolutionary loss, and the sister group relationships of the species characterized by that secondary loss are supported by characters other than the size of the oral papillae.

There are 2 nomenclatorial options if classifications that reflect, as much as possible, current estimates of evolutionary relationships are to be maintained. *Bunodera* can be considered monotypic, containing only *B. luciopercae,* and *B. mediovitellata, B. sacculata,* and *B. eucaliae* can be added to a genus containing *Culaeatrema inconstans,* or *Culaeatrema* can be considered a junior synonym of *Bunodera.* If the first option is chosen, the genus *Bunoderina* Miller, 1936, for which

the type species is *B. eucaliae,* has priority over *Culaeatrema.* I do not advocate the recognition of monotypic genera in a phylogenetic analysis if they can be avoided, so the second option is suggested, and *Culaeatrema* Lasee, Font, and Sutherland, 1988, is considered a junior synonym of *Bunodera* Railliet, 1896; consequently, *Culaeatrema inconstans* Lasee, Font, and Sutherland, 1988, becomes *Bunodera inconstans* (Lasee, Font, and Sutherland, 1988) n. comb.

ACKNOWLEDGMENTS

My thanks to Deborah McLennan for inviting me to collaborate on a study of sexual selection and parasitism in *Culaea inconstans.* Thanks also are due William Font for discussions and advice during the course of this study and preparation of this manuscript. Funds for this study were provided by operating grant A7696 from the Natural Sciences and Engineering Research Council (NSERC) of Canada.

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